Conclusions: The temporal coincidence of viral dispersions between countries and expansion of global H5N1 genetic diversity suggests the geographical spread might expand the ecological niche and species distribution for H5N1-HPAIV. Furthermore, our study suggests that the mutations on antigenic epitopes of H5N1-HPAIV are essential for their adaptation and immune evasion in bird populations, particularly at the early stage of invasion.

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Pathogenicity of Lagos Bat Virus - An African Rabies-Related Lyssavirus

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Lagos bat virus (LBV) constitutes genotype (gt) 2 in the Lyssavirus genus and the principal hosts are fruit bats. Members of this genus cause fatal rabies encephalitis. Based on phylogeny, serologic cross-reactivity and peripheral pathogenicity to mice, lyssaviruses were divided into two phylogroups. Phylogroup I viruses are pathogenic for mice when inoculated via the intracerebral (i.c.) and intramuscular (i.m.) routes. Phylogroup II viruses (LBV and Mokola virus (MOKV)) were shown to be pathogenic for mice only when inoculated via the i.c. route. This study compared the pathogenicity of several isolates of LBV in a murine model. Amino acid substitutions along the glycoprotein, previously suggested to be important for peripheral pathogenicity of lyssaviruses, were also analysed.

Four-week-old mice were inoculated with lyssavirus isolates using different routes of inoculation and different doses of inoculum. Mice were observed for 56 days. The direct fluorescent antibody test (FAT) was performed on mouse brain collected from succumbed or euthanized mice. The nucleotide sequence of pathogenic domains of LBV isolates was determined and amino acid sequences were compared using multiple alignments.

The peripheral pathogenicity of some representatives of LBV in the murine model were found to be as high as the corresponding pathogenicity of rabies virus. Domains on the glycoprotein that has previously been implicated in virulence, were found to differ between LBV strains that demonstrated a difference in pathogenicity.

Previous studies suggested that LBV were not pathogenic to mice when introduced peripherally. We demonstrated that representatives of LBV caused rabies in mice when introduced i.m and therefore the pathogenicity had been underestimated previously. The surveillance and public health precautions for LBV must be enhanced and this is particularly important since commercially available rabies biologicals do not protect against this virus.

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Design and Development of a Novel Electrochemical DNA Biosensor for Rapid Molecular Identification of *Enterococcus faecium*

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Enterococci have emerged as prominent nosocomial pathogens that cause a variety of clinical infections in many parts of the world over the last decade. The most common enterococci strains isolated from clinical samples are *E. faecium* and *E. faecalis*. Enterococci are known to have acquired resistance to vancomycin (glycopeptide) antimicrobials, resulting in the rapid increase of vancomycin resistant enterococci (VRE) strains in human. The conventional culture methods are time-consuming and laborious. Alternative molecular techniques polymerase chain reaction (PCR) and agarose gel electrophoresis utilize harmful elements such as carcinogenic ultraviolet light and ethidium bromide. In addition, optical-based techniques such as real-time PCR are expensive and require specialized equipments. Recently, interest has been increasing in the development of simple, inexpensive and disposable DNA biosensors for field and clinical assays. In the present study, an electrochemical DNA biosensor was designed and developed for detection of *E. faecium*. Design, fabrication and electrochemical characterization of screen-printed carbon electrodes (SPCEs) were carried-out. Optimization of the PCR and biosensor protocols such as PCR hapten labeling, washing step and peroxidase oxidation signal were performed. Under the optimized conditions, the oxidation signal threshold value was determined at 2.00 ± 0.02 μA. The analytical specificity of the biosensor assay was evaluated with reference *E. faecium* and non-*E. faecium* strains and was found to be 100%, while analytical sensitivity of the assay was 10 CFU/ml. The biosensor assay gave quantitative results rather then qualitative results when compared with agarose gel and DNA-chromatography based tests. In this study, the biosensor was optimized using *E. faecium* as a model organism and proved to be sensitive and specific. Hence in future, it will be possible to use this biosensor for antimicrobial resistant determinants, other microorganisms or mutant gene detection in hospital and environmental settings.

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